10/531303
JC13 Rec'd PCT/PTO 14 APR 2005

CARBOHYDRATE BASED ANTI-BACTERIALS

FIELD OF THE INVENTION

The invention relates to disaccharide compositions that have antibacterial properties.

BACKGROUND OF THE INVENTION

Bacteria have a great ability to generate resistance to drugs through lateral gene transfer, mutation of enzymes, or by expressing enzymes which actively pump out the drug or break it down. Over the past 10 years resistance to existing drugs has become a significant problem in many countries. No new antibacterial drugs have been developed over the past 15 years. Vancomycin is currently the drug of last resort to combat the multidrug resistant Gram-positive bacteria. In many places vancomycin-resistant Staphylococcus aureus and Enterococci (VRE) have been discovered. There is thus a desperate need for a new antibacterial drug to replace the drug of last resort.

There are a host of cytoplasmic targets for the development of new antibacterials, such as gyrase inhibitors, protein synthesis inhibitors, muramyl cascade inhibitors and many more. The major hurdle in designing such drugs is that in addition to enzyme based activity these drugs need to cross the bacterial cell wall to exert their antibacterial effect. On the other hand, enzymes involved in the stage III synthesis of the bacterial cell wall exist on the cell wall exterior, and therefore drugs inhibiting these enzymes can exert their bactericidal or bacteriostatic effect without having to cross the cell wall. Penicillin, cephalosporin and vancomycin are drugs that act on the transpeptidase enzymes which control the final steps in the peptidoglycan biosynthesis. Moenomycin is known to act on the transglycosylase enzymes, which are similarly involved in the polymerization of disaccharide precursors. Moenomycin displays very high potency at MIC level, and is used in animal feed as a growth promoter.

Moenomycin is a lipid-linked pentasaccharide. Through extensive SAR experiments it was realised that smaller fragments of moenomycin were capable of exerting antibacterial activity. Trisaccharide fragments of moenomycin still display antibacterial activity, but are not sufficiently stable to be useful drugs. On the basis of this, Sofia and coworkers discovered a new series of disaccharides, carrying aromatic

substituents in well defined positions around the disaccharide, which displayed significant MIC activity [WO0064915 and WO9926596].

A further class of disaccharide molecules, based on a sub-structure of vancomycin was shown to have antibacterial activity against vancomycin resistant bacteria. This class of molecules was subsequently demonstrated to contain transglycosylase inhibitors, and were not transpeptidase inhibitors as is vancomycin itself [WO9853813].

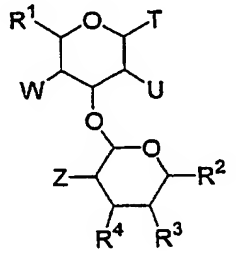
SUMMARY OF THE INVENTION

The present invention is directed to antibacterial compositions and is especially directed to a method of reducing bacterial growth by contacting bacteria with particular disaccharide like moieties.

The present invention may also be directed to an antibacterial pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of at least one particular disaccharide like moiety.

The present invention may also be directed to a method of screening such compounds for anti-bacterial activity by contacting the compounds with a Gram-positive or Gram-negative bacteria and monitoring the growth or growth inhibition of the bacteria.

In a first aspect, the invention provides a method of inhibiting bacterial growth by contacting a bacteria with at least one disaccharide compound of General Formula I,



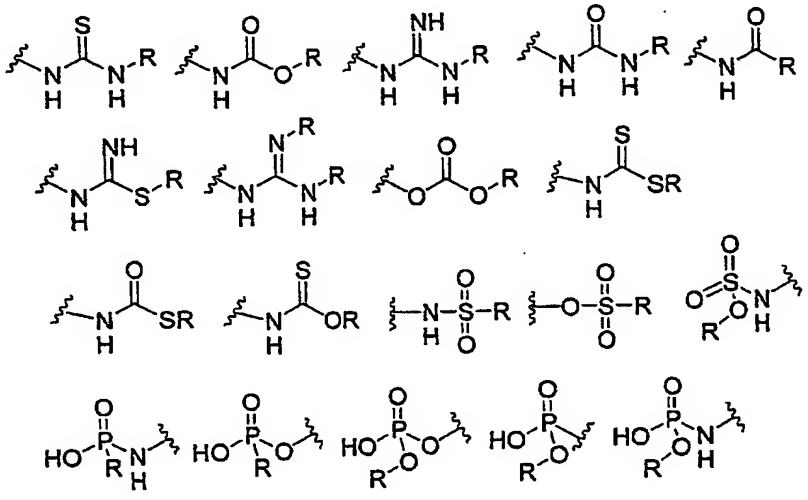
General Formula I

Wherein the pyranose rings may be of any configuration,

T is either R or -XR, where X is defined as oxygen, sulphur, NHC(O)-, and wherein R is selected from the non-limiting set comprised of H, or an alkyl, alkenyl, alkynyl,

heteroalkyl, aryl, heteroaryl, arylalkyl or heteroarylalkyl of 1 to 20 atoms which is optionally substituted, and can be branched or linear. Typical substituents include but are not limited to OH, NO, NO_2, NH_2, N_3, halogen, CF_3, CHF_2, CH_2F, nitrile, alkoxy, aryloxy, amidine, guanidiniums, carboxylic acid, carboxylic acid ester, carboxylic acid amide, aryl, cycloalkyl, heteroalkyl, heteroaryl, aminoalkyl, aminodialkyl, aminotrialkyl, aminoacyl, carbonyl, substituted or unsubstituted imine, sulfate, sulfonamide, phosphate, phosphoramide, hydrazide, hydroxamate, hydroxamic acid, heteroaryloxy, aminoalkyl, aminoaryl, aminoheteroaryl, thioalkyl, thioaryl or thioheteroaryl, which may optionally be further substituted,

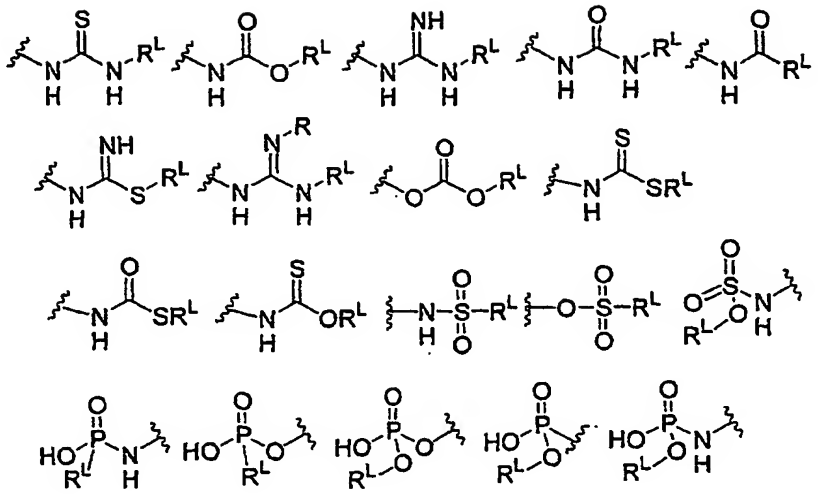
U and Z independently selected from OR, NHR, NR(R) (where R may be the same or different), or the following non-limiting set,



R^1 and R^2 are independently selected from H, CH_3, CH_2XR, and C(O)NHR,

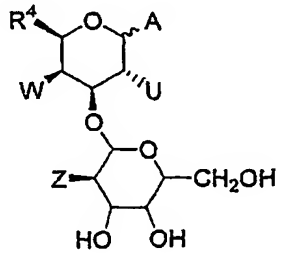
R^3 and R^4 are independently selected from H, OH, OR, NHCOR, and

W is independently selected from OR^L, NHR^L, NR^LR, or the following the following non-limiting set,



Wherein R^L is a substituted or unsubstituted, linear or branched, saturated or unsaturated C3 to C55 alkyl, heteroalkyl, arylalkyl, alkylaryl chain. Substituents may include but are not limited to acidic groups such as carboxylic acids, sulfonic acids, phosphoric acids, tetrazoles, or other carboxylic acid mimetics or basic groups such as amines, guanidines, amidines, imidazoles or other amine mimetics .

In a further aspect, the invention provides a method of inhibiting bacterial growth by contacting a bacteria with at least one disaccharide compound of General Formula II,



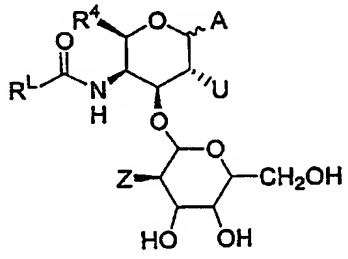
General Formula II

Wherein the disaccharide linkage is alpha or beta,

A is defined as hydrogen, OR or SR, and

R, U, W, Z and R^4 are defined as in General Formula I.

In a more preferred aspect, the invention provides a method of inhibiting bacterial growth by contacting a bacteria with at least one disaccharide compound of General Formula III,



General Formula III

Wherein A is defined as in General Formula I, and
U, Z, R^L and R^4 are defined as in General Formula I.

The bacterial may be Gram-positive or Gram-negative bacteria. The bacteria may comprise an *E-coli* bacteria, a Staphylococci Bacteria such as *Staphylococcus aureus*, or other bacteria such as *Micrococcus luteus* (ATCC272), *Staphylococcus aureus* (ATCC29213), *Staphylococcus aureus* (ATCC43300) MRSA, *Enterococcus faecalis* (ATCC29212), *Enterococcus faecalis* (ATCC51299) Vancomycin resistant and *Streptococcus pyogenes* (ATCC8668).

The method may comprise administering an effective amount of a compound of the first aspect, to a subject in need of such treatment. The subject may be a human, or may be a domestic, companion or zoo animal.

In another form, the invention may reside in an antibacterial composition comprising at least one compound as described above. The composition may comprise a pharmaceutical composition.

The compounds of the invention may be mixed with a pharmaceutical acceptable carrier, adjuvant, or vehicle which may comprise a-toxic carrier, adjuvant, or vehicle that may be administered to a patient, together with a compound of this invention, and which does not destroy the pharmacological activity thereof.

The pharmaceutical derivative may comprise a salt, ester, salt of an ester or other derivative of a compound of this invention which, upon administration to a recipient, is capable of providing, either directly or indirectly, a compound of this invention, although no limitation is meant thereby.

Compounds of the invention may be administered orally such as by means of a tabled, powder, liquid, emulsion, dispersion and the like; by inhalation; topically such as by means of a cream, ointment, salve etc; and as a suppository, although no limitation is meant thereby.

Methods and pharmaceutical carriers for preparation of pharmaceutical compositions are well known in the art, as set out in textbooks such as Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Company, Easton, Pennsylvania, USA.

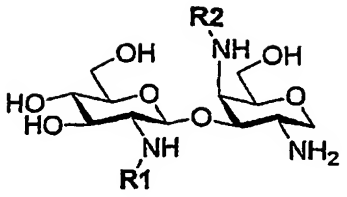
It will be clearly understood that, if a prior art publication is referred to herein, this reference does not constitute an admission that the publication forms part of the common general knowledge in the art in Australia or in any other country.

BEST MODE

MIC testing:

The broth microdilution format of the National Committee for Clinical Laboratory Standards (NCCLS) approved standard for susceptibillity tests as outlined in M7-A4 "methods for dillution Antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard – fifth edition", January 2000 was utilized for minimum inhibitory concentration testing in Mueller-Hinton broth. The broth for *Streptococcus pyogenes* testing was supplemented with 2% laked horse blood. A positive result in initial testing was determined by complete inhibition of macroscopic bacterial growth at a concentration of 128 micrograms per mL after incubation for 16 to 24 hours at 37 degrees C. In the case of *Micrococcus luteus*, incubation was at 30 degrees C.

Example 1



Comp. No.	R1	R2	Mass	R_f	SA24	SA48	EC24
1	A5	A9	679	4.62	+	n.d.	-

In all examples, + indicates an MIC value of less than 128 micrograms per mL, - indicates an MIC of greater then 128 micrograms per mL and n.d. indicates not determined.

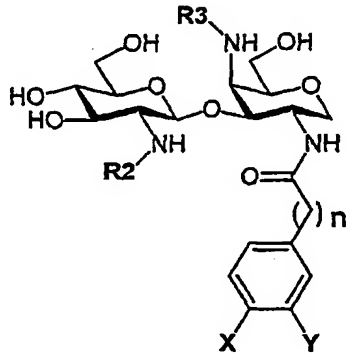
Bacterial Types are:

SA24 S. aureus after 24 hours exposure

SA48 S.aureus after 48 hours exposure

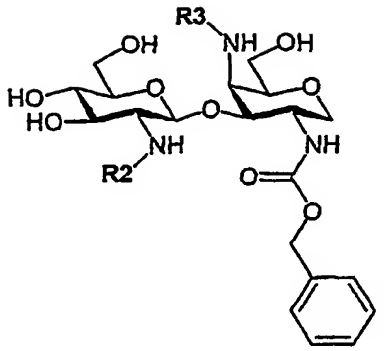
EC24 E. coli after 24 hours exposure

Example 2



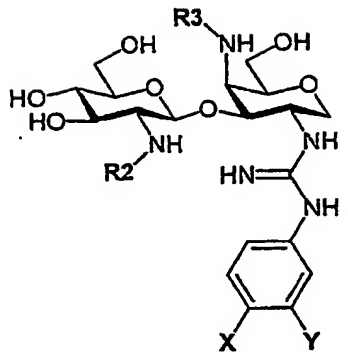
Comp. No.	n	X	Y	R2	R3	MS	R_f	SA24	SA48	EC24
2	1	A1	A10	A11	A7	875	n.d	+	+	-
3	1	A1	A10	A4	A9	831	n.d	+	+	-
4	0	A1	A10	A12	A9	800	5.1	+	n.d	-
5	0	A1	A10	A5	A7	862	4.92	+	+	-
6	0	A1	A10	A5	A9	851	5.36	+	n.d	n.d.
7	1	A10	A1	A5	A7	876	5.01	+	+	-

Example 3



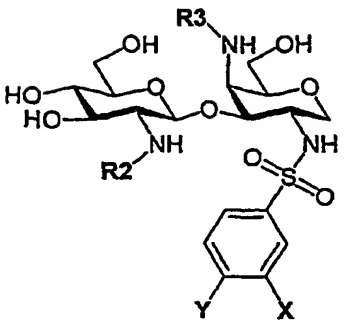
Comp. No.	R2	R3	MW	R_f	SA24	SA48	EC24
8	A5	A7	824	4.72	+	+	-
9	A5	A9	813	5.56	+	n.d.	n.d.

Example 4



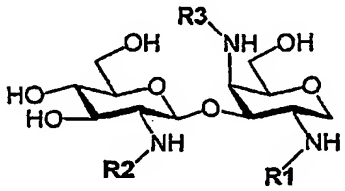
Comp. No.	X	Y	R2	R3	MW	Rf	SA24	SA48	EC24
10	A1	A10	A12	A7	875	n.d.	+	+	-
11	A1	A10	A4	A9	831	5.18	+	+	+
12	A1	A10	A4	A7	843	4.65	+	+	+
13	A1	A10	A4	A1	663	3.2	+	n.d.	n.d.
14	A1	A10	A5	A9	864	5.27	+	+	-
15	A1	A10	A19	A9	863	4.85	+	+	+
16	A1	A10	A19	A7	875	4.23	+	+	+
19	A1	A10	A19	A25	849	4.8	+	+	+
20	A1	A10	A19	A22	861	3.49	+	+	+
21	A1	A10	A19	A16	889	3.57	+	+	+
22	A1	A10	A19	A23	930	5.1	+	+	+
23	A1	A10	A19	A26	831	3.56	+	+	+
24	A1	A10	A19	A27	899	4.22	+	+	+
25	A1	A10	A19	A28	904	3.3	+	+	+
26	A1	A10	A19	A29	918	3.5	+	+	+
27	A14	A1	A2	A9	800	5.01	+	n.d.	+
28	A14	A1	A3	A9	762	4.81	+	n.d.	+
29	A14	A1	A12	A9	764	4.92	+	n.d.	+
30	A14	A1	A4	A9	782	4.9	+	n.d.	+
31	A14	A1	A15	A9	765	5.4	+	n.d.	+

Example 5



Comp. No.	X	Y	R2	R3	MW	Rf	SA24	SA48	EC24
36	A10	A1	A17	A7	919	n.d.	+	+	n.d.
37	A10	A1	A5	A7	898	4.99	+	+	-
38	A1	A13	A2	A9	818	5.56	+	n.d.	n.d.
39	A1	A13	A5	A7	844	4.72	+	+	-
40	A1	A13	A5	A9	833	5.63	+	n.d.	-

Example 6



Comp.	R1	R2	R3	MW	Rf	SA24	SA48	EC24
42	A20	A20	A8	963	n.d.	+	n.d.	n.d.
43	A5	A1	A7	690	n.d.	+	n.d.	n.d.
44	A5	A3	A7	824	n.d.	+	+	n.d.
45	A5	A3	A1	644	3.82	+		n.d.
46	A5	A21	A7	822	4.73	n.d.	+	-
47	A5	A21	A1	642	3.39	+	n.d.	n.d.
48	A5	A17	A7	898	n.d.	+	+	-
49	A5	A4	A7	844	4.9	n.d.	+	-
50	A5	A4	A1	664	3.8	+	n.d.	n.d.

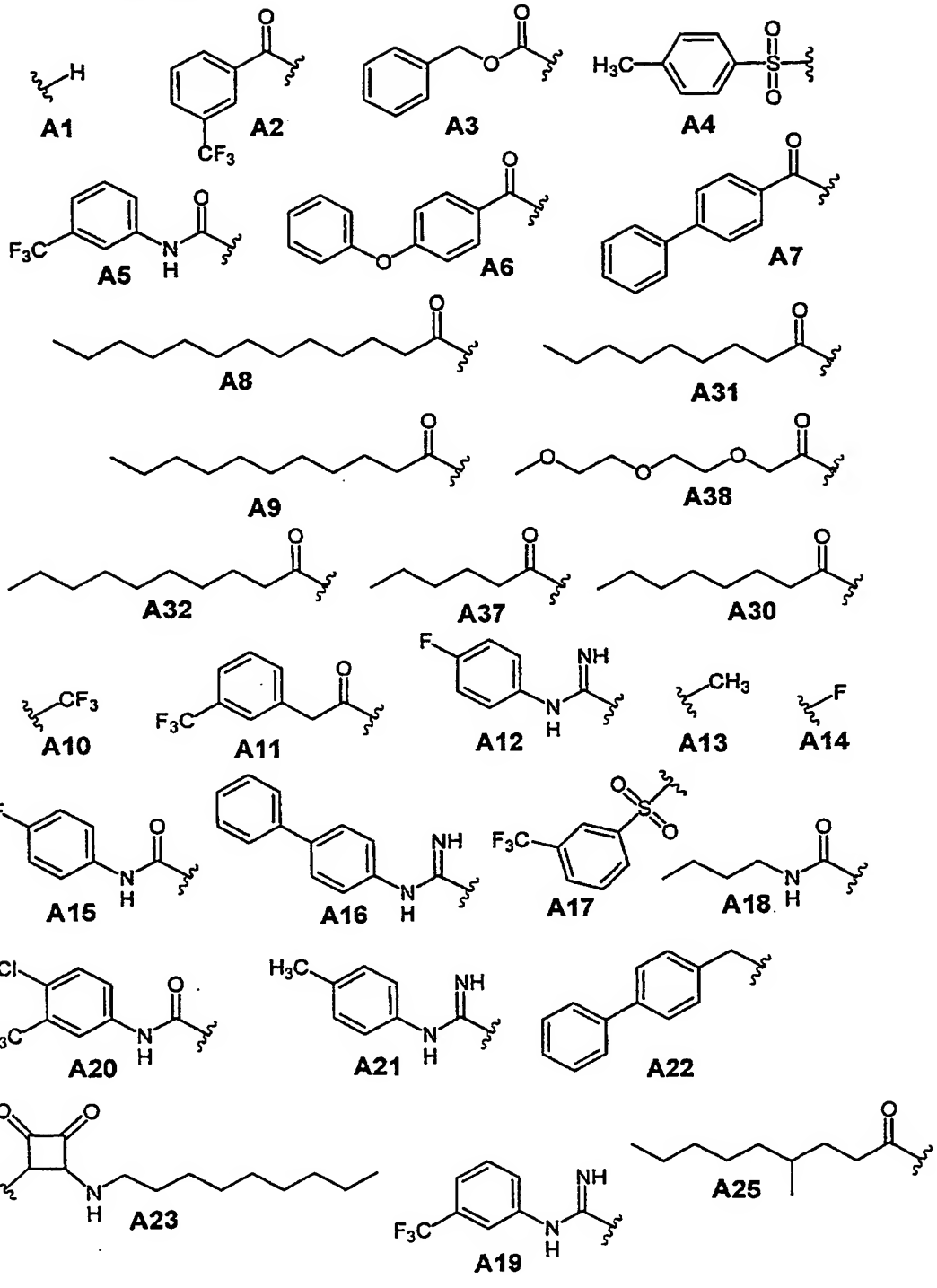
51	A5	A4	A9		n.d.	n.d:	n.d.	n.d.
52	A5	A44	A7	823	3.98	n.d.	+	-
55	A5	A5	A25	851	5.47	+	+	n.d.
56	A5	A5	$C_{10}H_{21}$	837	5.38	+	+	n.d.
57	A5	A5	A39	857	4.9	+	+	n.d.
58	A5	A5	A40	861	5.01	+	+	n.d.
59	A5	A5	A22		n.d.	+	+	-
60	A5	A5	bis-pentyl	837	4.9	+	+	n.d.
61	A5	A5	A32	851	5.56	+	+	n.d.
62	A5	A5	A31	837	5.08	+	+	n.d.
63	A5	A5	A30	823	5.1	+	+	n.d.
64	A5	A5	A33	929	5.82	+	+	n.d.
65	A5	A5	A34	942	5.17	+	+	n.d.
66	A5	A5	A41	938	4.81	-	n.d.	n.d.
67	A5	A5	A42	952	4.89	-	n.d.	n.d.
68	A5	A5	A32	901	5.36	-	n.d.	n.d.
69	A5	A5	A36	901	5.45	+	n.d.	n.d.
70	A5	A5	A37	795	4.62	-	n.d.	n.d.
71	A5	A5	A46	880	4.62	-	n.d.	n.d.
72	A5	A5	A47	880	4.81	-	n.d.	n.d.
73	A5	A5	A6	893	5.1	+	n.d.	n.d.
74	A5	A5	A7	877	4.99	+	n.d.	n.d.
75	A5	A5	A23	932	5.63	+	n.d.	n.d.
76	A5	A5	A8	893	6.09	+	n.d.	n.d.
77	A5	A5	A9	865	5.63	+	+	-
78	A5	A3	A9	813	5.45	+	n.d.	n.d.
79	A5	A4	A9	833	5.73	+	n.d.	n.d.
80	A18	A4	A9	744	n.d.	+	n.d.	n.d.

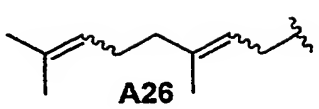
The following compounds were tested against additional organisims with the following results.

1. *Micrococcus luteus* (ATCC272)
2. *Staphylococcus aureus* (ATCC29213)
3. *Staphylococcus aureus* (ATCC43300) MRSA
4. *Enterococcus faecalis* (ATCC29212)
5. *Enterococcus faecalis* (ATCC51299) Vancomycin resistant
6. *Streptococcus pyrogenes* (ATCC8668)

Compound	1	2	3	4	5	6
76	+	+	+	+	+	+
42	+	+	+	+	+	+
75	+	+	+	+	+	+
68	+	+	-	+	-	+
65	+	-	-	+	-	+
69	+	+	+	+	-	+
70	+	-	-	+	-	+
73	+	+	+	+	+	+
74	+	+	+	+	+	+
66	-	-	-	-	-	+
67	+	+	+	+	+	+
77	+	+	+	+	+	+
51	+	+	+	+	+	+
56	+	+	+	+	+	+

TABLE 1 *Side Arms*





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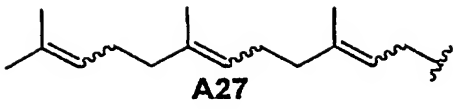

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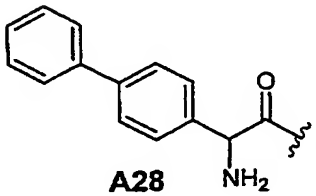

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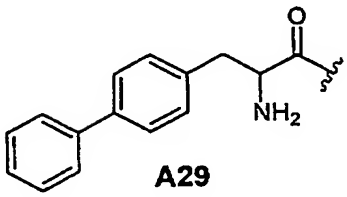

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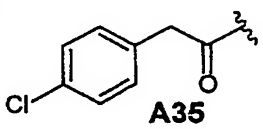

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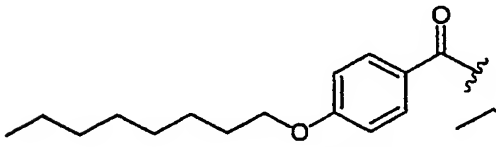

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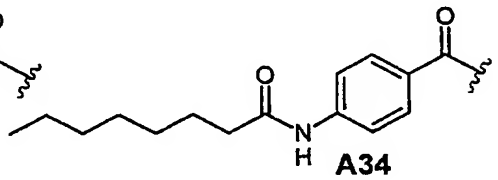

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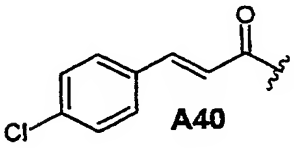

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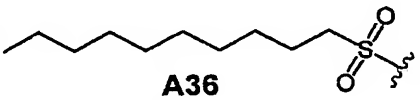

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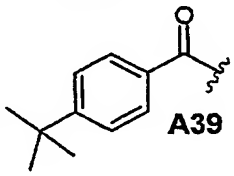

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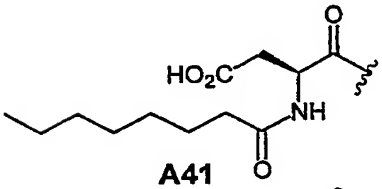

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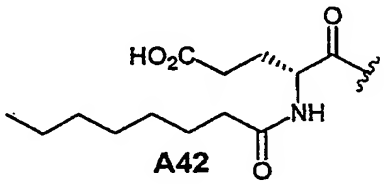

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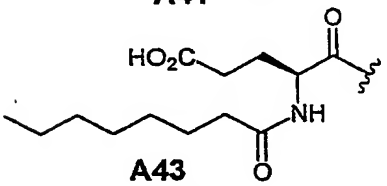
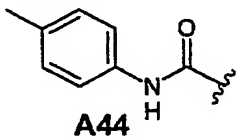
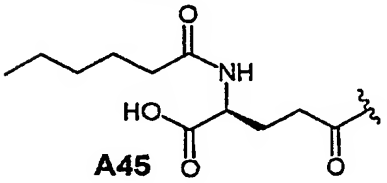


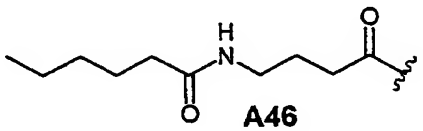
A43



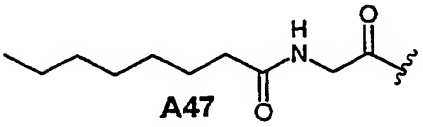
A44



A45



A46



A47

Throughout the specification and the claims unless the context requires otherwise, the term "comprise", or variations such as "comprises" or "comprising", will be understood to apply the inclusion of the stated integer or group of integers but not the exclusion of any other integer or group of integers.

It should be appreciated that various other changes and modifications can be made to any embodiment described without departing from the spirit and scope of the invention.